Appl. No. 09/857,433
Amendment dated: April 20, 2005
Reply to OA of: October 20, 2004

REMARKS

Applicants have amended the specification and claims to more particularly define the invention taking into consideration the outstanding Official Action. The specification has been amended to cross reference the related applications and to add standard headings as requested in the Official Action. Also, a brief description of the drawings has been added, new Figure 4 and a new Abstract has been added without legal terminology. All of these amendments are fully supported by the application as originally filed.

All of the claims have been canceled from the application without prejudice or disclaimer and new claims 24-43 have been added in an effort to address the rejection on the grounds of indefinity spanning pages 5 and 6 of the Official Action. Specifically, the new claims now explicitly recite steps of contacting a sample with an enzyme, generating a signal, assessing this signal and relating the assessed signal to the concentration to be assayed. The additional process steps now recited in the claims are fully supported by the specification and claims as originally filed and in particular upon the sequence of steps presented on pages 15-16 and the assay protocols given in Examples 2 and 3.

The rejection of claims 1-15, 18, 19, 21-23 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention has been carefully considered but is most respectfully traversed.

Each of the terms objected to by the Examiner has been removed, corrected or clarified and consistent terms have been used throughout the claims for the biological fluid samples. These amendments are all fully supported by the application as originally

filed as would be appreciated by one of ordinary skill in the art to which the invention pertains. Applicants most respectfully submit that all the claims now present in the application are in full compliance with 35 U.S.C. §112 and are clearly patentable over the references of record.

Applicants enclose a flow diagram relating to the features on pages 15 and 16, as recommended by the Examiner. The flow diagram has not been added to the specification at this time and is submitted only to facilitate an understanding of the invention. The flow diagram indicates the various possible steps in the homocysteine assay method. The improvements embodied in various claims have been emphasized by use of different boxing and bracketing shapes. Specifically, the addition and subsequent removal of a reducing agent, as recited in new claim 24, relates to the steps in angled (brackets and boxes, the use of a fluid generated from lyophilised homocysteine desulfurase (HDS), as indicated in new claims 25 and 26, is presented in curled brackets) and the pyruvate removal method described in claim 27 is indicated in curved brackets/boxes.

The rejection of claims 1, 3, 5 and 19 under 35 U.S.C. 103 as unpatentable over Coombs has been carefully considered but is most respectfully traversed.

Applicants wish to direct the Examiner's attention to the basic requirements of a prima facie case of obviousness as set forth in the MPEP § 2143. This section states that to establish a prima facie case of obviousness, three basic criteria first must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Section 2143.03 states that all claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Applicants also most respectfully direct the Examiner's attention to MPEP § 2144.08 (page 2100-114) wherein it is stated that Office personnel should consider all rebuttal argument and evidence present by applicant and the citation of In re Soni for error in not considering evidence presented in the specification. In this regard, see especially figures 1, 2 and 3 and the discussion on pages 17 and 18 of Applicants' specification.

The rejection of claims 1, 3, 5 and 19 as obvious under 35 USC 103 over Coombs has been carefully considered but is most respectfully traversed, The new claims have been reformatted to emphasize the contribution of the present invention over the prior art. In particular, the present invention allows the improvement of previously known homocysteine assay methods by reduction in the background signal and improvement of the signal to noise ratio. This in turn provides an assay with greater sensitivity and accuracy. This is indicated in the last two full paragraphs of page 1 of the application as filed and is now embodied quite clearly in the independent claims.

Specifically in claim 1 (now new claim 24), the Examiner believes that claim 1 is rendered obvious by Coombs on the basis that a DTT reducing agent is employed and that in the subsequent change of pH, during the assessment of NAD, at least some

destruction of DTT would be expected. This method would, however, not provide the improvement now demonstrated in the present application since the reduction noise results from removing the DTT reducing agent after conversion by HDS but before generating a signal corresponding to the homocysteine conversion product (in this case alpha-ketobutyrate). The presently claimed invention has established that the presence of a reducing agent such as DTT, while essential at the HDS reaction stage causes increased background noise at later stages of the assay and therefore effective removal of this agent after the HDS reaction step provides the claimed improvement. This is set out in claim 1 (new claim 24) but is in no way taught towards in the Coombs reference. Furthermore, Coombs would not inherently have caused this improvement since any destruction of DDT brought about by the method of Coombs occurs too late in the assay process to give reduced background noise.

The Examiner then objects to claim 3 (new claim 26) on the basis that it is well known to store enzymes with reducing agents and stabilizers and thus to add a liquid reagent containing these components into the assay would, the Examiner believes, be obvious. Claim 3 (new claim 26) as herewith enclosed has been made dependent upon claim 2 (new claim 25) such that the liquid reagent must be made by adding liquid to a lyophilisate containing the enzyme and a thiol-free protectant. This is believed to be new and non-obvious, as discussed below, and thus new claim 26 is also believed acceptable.

With regard to claim 5 (new claim 28), the Examiner objects to "removing the sample", as recited previously, since the homocysteine would be removed by the enzymatic activity of HDS. In fact, new claim 28 relates to the embodiment of the invention described in the section bridging pages 12 and 13 of the specification as filed, in which an inactivated enzyme is described. By use of this inactivated enzyme, the homocysteine can be bound and removed from the remainder of the sample prior to

reaction. This removal step prevents interference by other material in the sample in any of the subsequent stages of the homocysteine assay and thereby improves the sensitivity and reliability. This assay method is now more clearly recited in enclosed new claim 28, which is based on the passage bridging pages 12 and 13.

Since there is no prior art indicating that use of an inactivated HDS to bind and separate homocysteine might improve a homocysteine assay method, Applicants believe that this claim is evidently new and non-obvious. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 4, 6 and 18 under 35 U.S.C. 103(a) as being unpatentable over Tan has been carefully considered but is most respectfully traversed.

In particular, Tan proposes an assay for homocysteine in which H2S produced from reaction of both cysteine and homocysteine is detected and this result is then corrected by subtraction of the result from an assay for pyruvate, which is produced in reaction of cysteine. The key difference in the present application is that the pyruvate to be removed is that present before any reaction with HDS takes place. This prior pyruvate removal improves the signal noise ratio of the assay but is quite different from the pyruvate reaction in Tan. In Tan, the pyruvate being measured should, in order to give an accurate result, only be that generated in the reaction with cysteine.

In addition to the above, claims 4 and 6 (new claims 27 and 29) now explicitly recite the homocysteine conversion product as being alpha-ketobutyrate. As will be clear to the Examiner and a worker of normal skill in the art, the reaction of cysteine with HDS cannot form alpha-ketobutrate (since cysteine has one methylene group too few and would thus form pyruvate), and therefore the "correction factor" method of Tan could not possibly be applied to a method in which alpha-ketobutrate was used as the homocysteine conversion product. The method of Tan can only be used when H2S is

measured and cannot therefore teach towards the methods of claims 4 and 6 (new claims 27 and 29).

The inventive step apply to claim 4 (new claim 27) applies equally to claim 6 (new claim 29) since the method of pyruvate removal has no effect upon the resulting improvement in signal noise. Size exclusion filtration as now recited in claim 6 is well know to the skilled worker, but the application of this method to improve the accuracy and sensitivity of a homocysteine assay has never previously been indicated. Accordingly, it is most respectfully requested that this objection be withdrawn.

The Examiner makes no rejection of claim 2 (new claim 25) on the grounds of either anticipation or obviousness and this claim is thus believed acceptable. In particular, there is no prior art that could indicate any advantage in the use of non-thiol protectants in the storage of HDS. Applicants have now established that this method improves the signal to noise in a HCy assay, as recited in new claim 25.

The rejection of claims 7, 8, 9, 11 and 21-23 under 35 U.S.C. 103(a) as being unpatentable over each of Coombs and Tan or the combination of Coombs in view of Tan has been carefully considered but is most respectfully traversed.

The Examiner objects to the remaining claims on the basis that these are obvious in view Coombs, Tan or the combination of Coombs in view of Tan. Each of these claims has now been placed in dependent form, depending upon one of the independent claims discussed above. Since each of these claims is novel and inventive it is believed that all dependent claims must correspondingly satisfy this requirement. Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants submit herewith a Revocation of Power of Attorney and Appointment of New Attorney in connection with this application and request all future correspondence be directed to the undersigned attorney.

Appl. No. 09/857,433
Amendment dated: April 20, 2005
Reply to OA of: October 20, 2004

In view of the above comments and further amendments to the specification and claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

BACON & THOMAS, PLLC

By: Richard E. Fichter
Richard E. Fichter
Registration No. 26,382

625 Slaters Lane, 4th Fl.
Alexandria, Virginia 22314
Phone: (703) 683-0500
Facsimile: (703) 683-1080

REF:kdd
A01.wpd

February 21, 2006

"NEW"

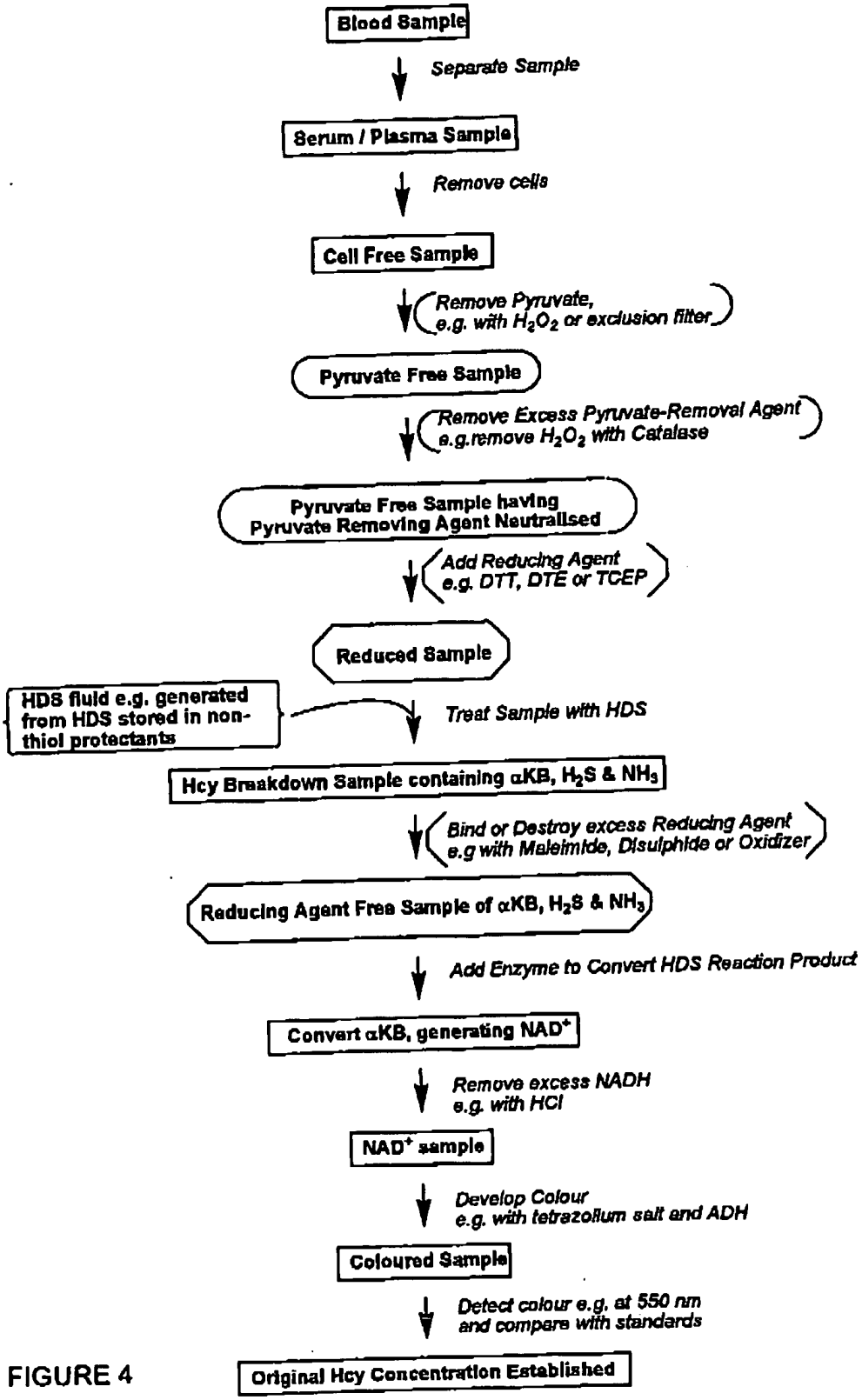
Blood Sample
Separate Sample
Serum / Plasma Sample
Remove cells
Cell Free Sample
Remove Pyruvate, e.g. with H_2O_2 or exclusion filter
Pyruvate Free Sample
Remove Excess Pyruvate-Removal Agent e.g.remove H_2O_2 with Catalase
Pyruvate Free Sample having Pyruvate Removing Agent Neutralised
Add Reducing Agent e.g. DTT, DTE or TCEP
Reduced Sample
HDS fluid e.g. generated from HDS stored in non-thiol protectants
Treat Sample with HDS
Hcy Breakdown Sample containing αKB, H_2S & NH_3
Bind or Destroy excess Reducing Agent e.g with Maleimide, Disulphide or Oxidizer
Reducing Agent Free Sample of αKB, H_2S & NH_3
Add Enzyme to Convert HDS Reaction Product
Convert αKB, generating NAD^+
Remove excess NADH e.g. with HCl
NAD^+ sample
Develop Colour e.g. with tetrazolium salt and ADH
Coloured Sample
Detect colour e.g. at 550 nm and compare with standards
Original Hcy Concentration Established

FIGURE 4